

Dear editor:

Thanks a lot for having reviewed our manuscript. We have revised the manuscript according to your suggestions. All of the revisions we make to the revised manuscript have been highlighted in our updated version of the manuscript. Some supporting materials have been uploaded with PDF or mp3 format. And our point to point answers to all reviewers are as follows.

### **Reviewer 1**

Interesting paper. QF granules have used in Chinese Medicine since antiquity. The major effect of this natural drug is an antiinflammatory action. This antiinflammatory action, inevitably, brings to cell Death and arrest of RNA transcripion in vitro. The paper deserves publication, in times when the general public is looking for "natural remedies" and probably it will have a major marketing if well publicized. Probably, the major effect of QF granules on cancer patients will be an antiinflammatory action plus a placebo effect, which should not be underevaluated. Concernibng the study, clearly the action should be analyzed in vivo. In the vitro study I would invite to add specific substance which could neutralize the antiinflammatory action of QF granules, to verify if QF granules have a direct action on cell cycle. In those days, we have to open our mind to new therapeutic possibilities. In this scenario to test an old traditional Chinese medicine, which has shown no side effects and a clear antiinflammatory action is Worthwhiile. However, it is long way to show an effective anticancer action of the QF granuels. The Auhtors should be encouraged to continue their studies. I would like to suggest to modify the title of the paper, adding at the end word "An hypothesis".

**Answer:** Thanks for your comments. We agree with your idea that animal study is very important to evaluate the mechanism of QFG on CRC growth, so we are going to establish the research plan for relevant animal experiment. And the study of the relationship between anti-inflammatory effects of QFG with tumor growth is a good

suggestion, we will arrange related experiments in animal experiments according to your suggestion. In addition, you suggested that we add "An hypothesis" to the title, but one of the requirements of this journal is that our title should be no more than 12 words, and we have simplified our title, so this suggestion may not be realized.

## **Reviewer 2**

In this manuscript, the authors examined the effect of Qingjie Fuzheng granule (QFG) in human colorectal cancer HCT-116 and HCT-8 cells. QFG decreased cell viability, inhibited cell proliferation and induced apoptosis. QFG treatment suppressed phosphorylated levels of PI3K, AKT and ERK. Although the results shows the anticancer effect of QFG clearly, I have several comments.

1. It's not clear whether the effect of QFG is selective for cancer cells or whether it's just toxic effect. The effect of QFG on non-malignant intestinal cells should be compared. QFG is used as a traditional Chinese medicine formula for patients. Animal experiments, such as a murine xenograft model of human colon cancer cells, will prove the anticancer effect of QFG.

**Answer:** Thanks for your advice. The compare of the effect of QFG on CRC cells with non-malignant intestinal cells is a very good suggestion. But due to the limitation of experimental conditions, the related experiments on non-malignant intestinal cells cannot be carried out at present (Once the experimental conditions permit, we will conduct this experiment immediately). It should be noted that we had conducted animal experiment on other project to evaluate whether QFG has toxic and side effects on animal body weight, intestinal tissue, liver and other organs, the result demonstrated that QFG has no toxic and side effects on animal body weight, intestinal tissue, liver and other organs compared with control group, which will be published as a paper. In addition, our in vivo study of subcutaneous xenotransplanted tumor model of human CRC in nude mice is in process.

2. Please provide information of plasma concentrations of QFG in human patients and

compare them to the concentrations used in this experiment.

**Answer:** Thanks for your suggestion. Basic experimental research cannot be equated with clinical application, the aim in this study is to elucidate the mechanism of QFG on CRC cells through in vitro experiments, so the concentrations of QFG used in this study were not compared with the plasma concentrations of QFG in human patients. But in our in vivo study which is in the process, the dosage of QFG used in animals is converted according to the clinical dosage of QFG in human patients.

3. QFG consists of a mixture of four herbs as shown in Table 1. The effect of QFG should be compared to those of single component, combination of two, and combination of three.

**Answer:** Thanks for your suggestion. Your suggestion will be developed as a new research project for us.

4. Treatment with QFG induced apoptosis. Why weren't cells of sub G1 phase observed in Figure 2?

**Answer:** Thanks for your comment. Sub G1 phase only occurs when DNA in cells breaks into small fragments. Although our result showed that QFG can promote CRC cells apoptosis, and in our cell cycle assays, it's certain that apoptosis has already occurred in CRC cells, but the DNA in cells has not broken into small fragments, so the sub G1 phase has not yet appeared.

### **Reviewer 3**

ESPS Manuscript NO: 44659 Title: Quingjie fuzheng granules inhibited proliferation and induced apoptosis by suppressing the PI3K/AKT and ERK signaling pathway in colorectal cancer cells This very interesting investigation include appliance of TRC in the treatment of colorectal cancer. The authors detailed described the effect of Quingjie fuzheng granules on the two type of colorectal cancer cells reveling the underlying mechanism that may provide benefit to the patient in the colorectal cancer

treatment. Title: It reflects the major topics and contents of the study. Abstract: It gives a clear delineation of the research objective and the results. Material and methods: The study design and methods are well described; appropriate statistical methods are selected and made. Results & Discussion: The data are clearly presented and documented; every results are elaborated in the discussion section. Figures: Reflects the major findings; suggesting the short description of main result in figure legend. In conclusion, this is a very interesting research, which bring the new approach to treatment of colorectal cancer with appliance of TCM.

**Answer:** Thanks a lot for your review.